# The Mechanisms of Arsenic bioremediation from water by the Green Microalgae *Chlorella vulgaris*


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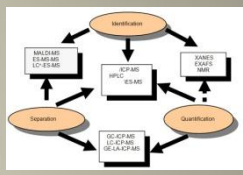
In collaboration with



**TESLA**
**Trace element Speciation Laboratory Aberdeen**

Leonardo Pantoja, Diane Purchase, Huw Jones, Jörg Feldmann and Hemda Garelick

# OUTLINE

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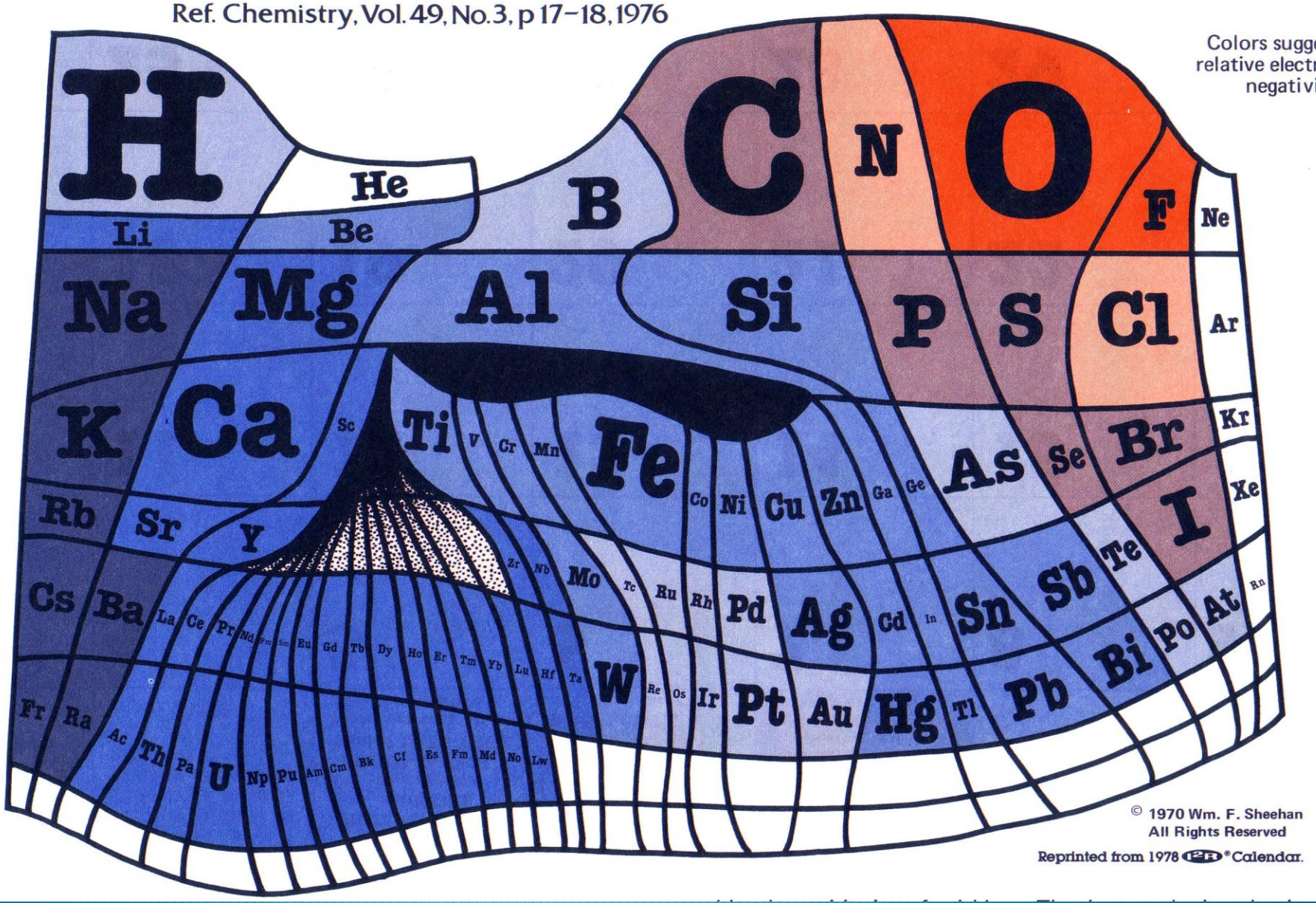
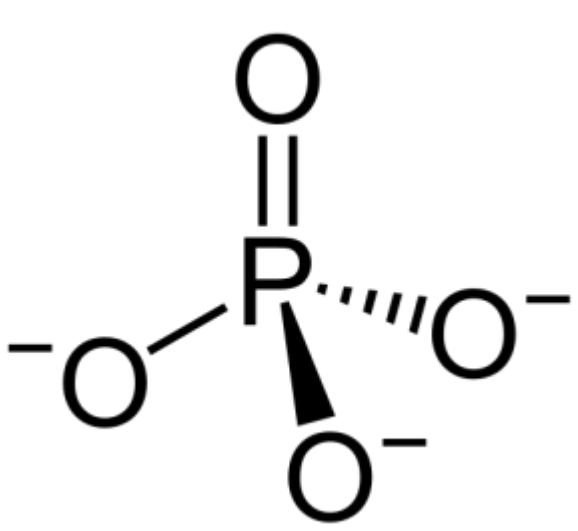
- Toxicity of arsenic to *C. vulgaris*
  - ASTM E1218 and flow cytometry
- Focused sonication for extraction for As-phytochelatin complexes
- Formation of As-GS/PC complexes as detoxification mechanism
  - Exposure to As(III) (Sodium arsenite)
  - Exposure to DMA(V) (Dimethylarsinic acid)
  - Exposure to As(V) (Sodium arsenate)
- Total As-GS/PC formation
- Transport of As-GS/PC to vacuoles
- Conclusions

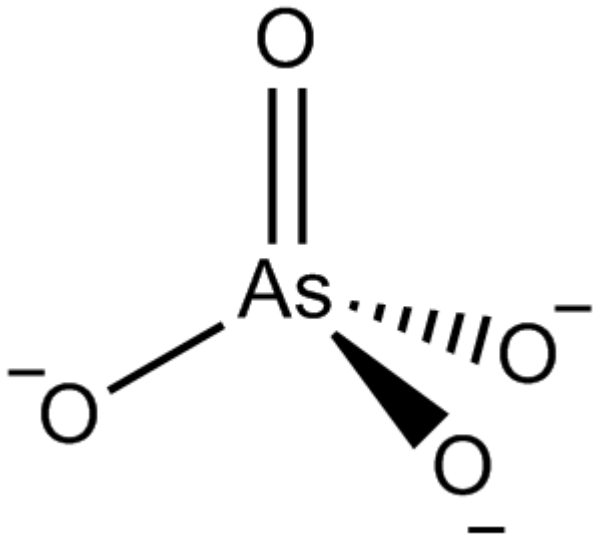
# The Elements According to Relative Abundance

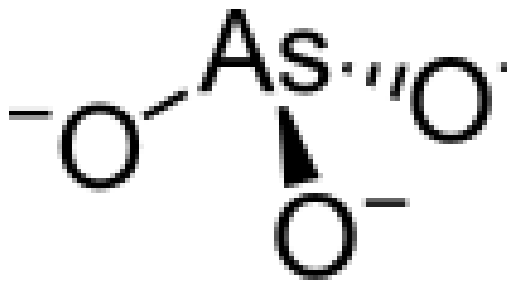
A Periodic Chart by Prof. Wm. F. Sheehan, University of Santa Clara, CA 95053
Ref. Chemistry, Vol. 49, No. 3, p 17–18, 1976

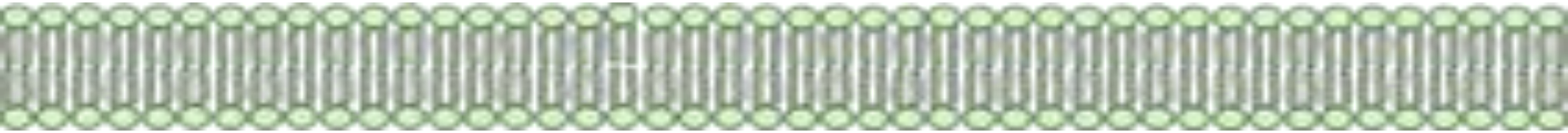


# Arsenic toxicity (similarities)

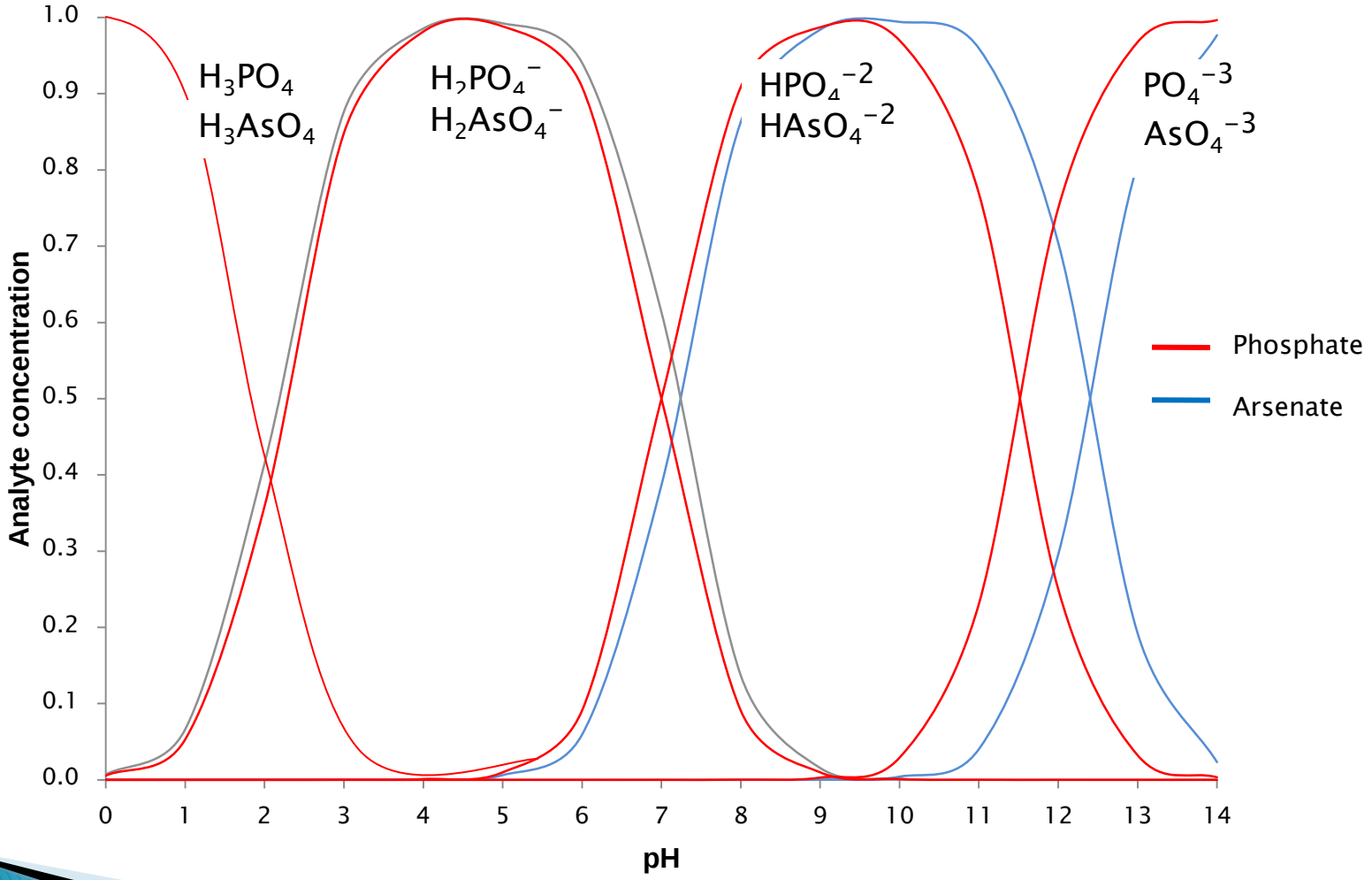


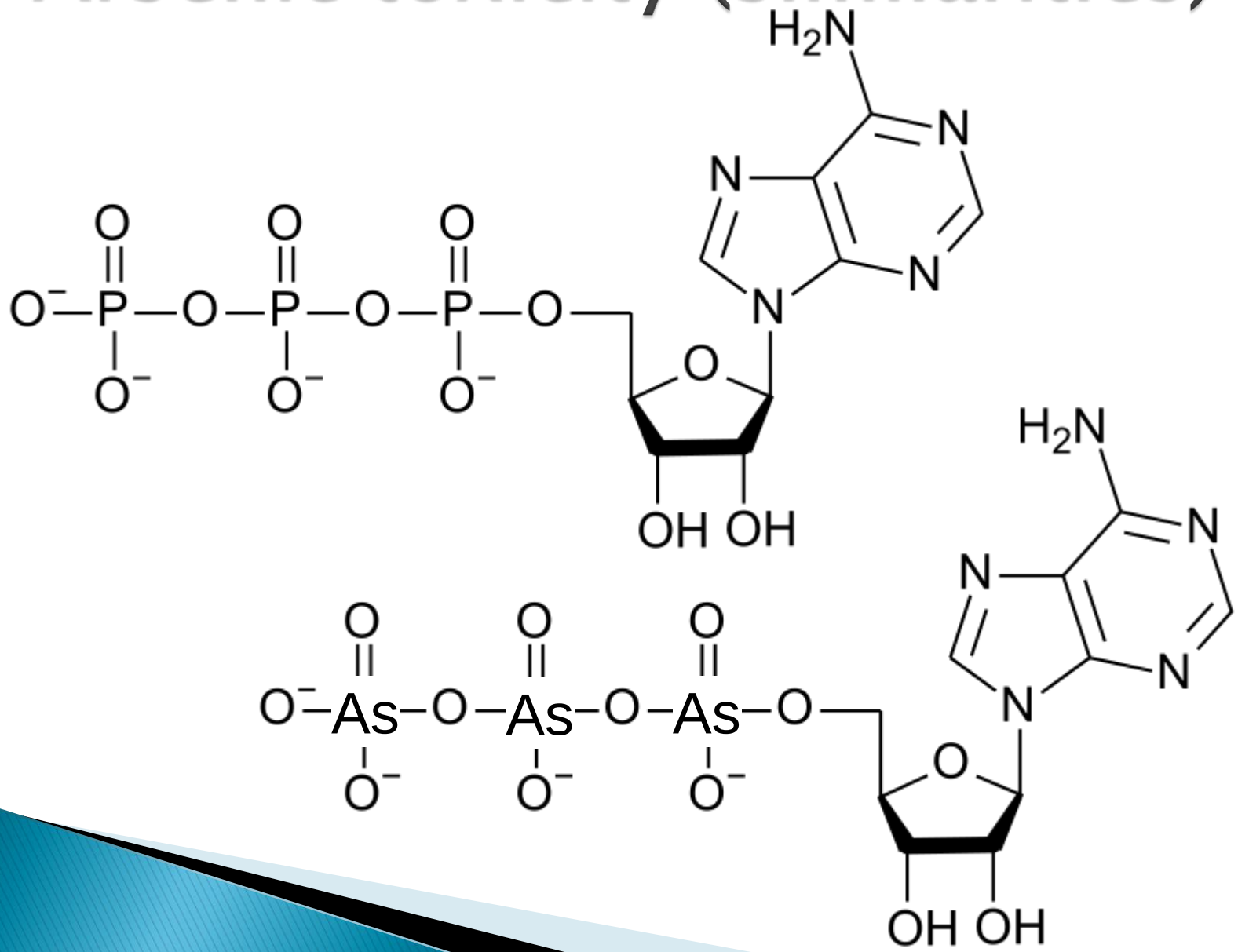



# Arsenic toxicity (similarities)



# Arsenic toxicity (similarities)



# TOXICITY OF ARSENIC TO *C. VULGARIS*

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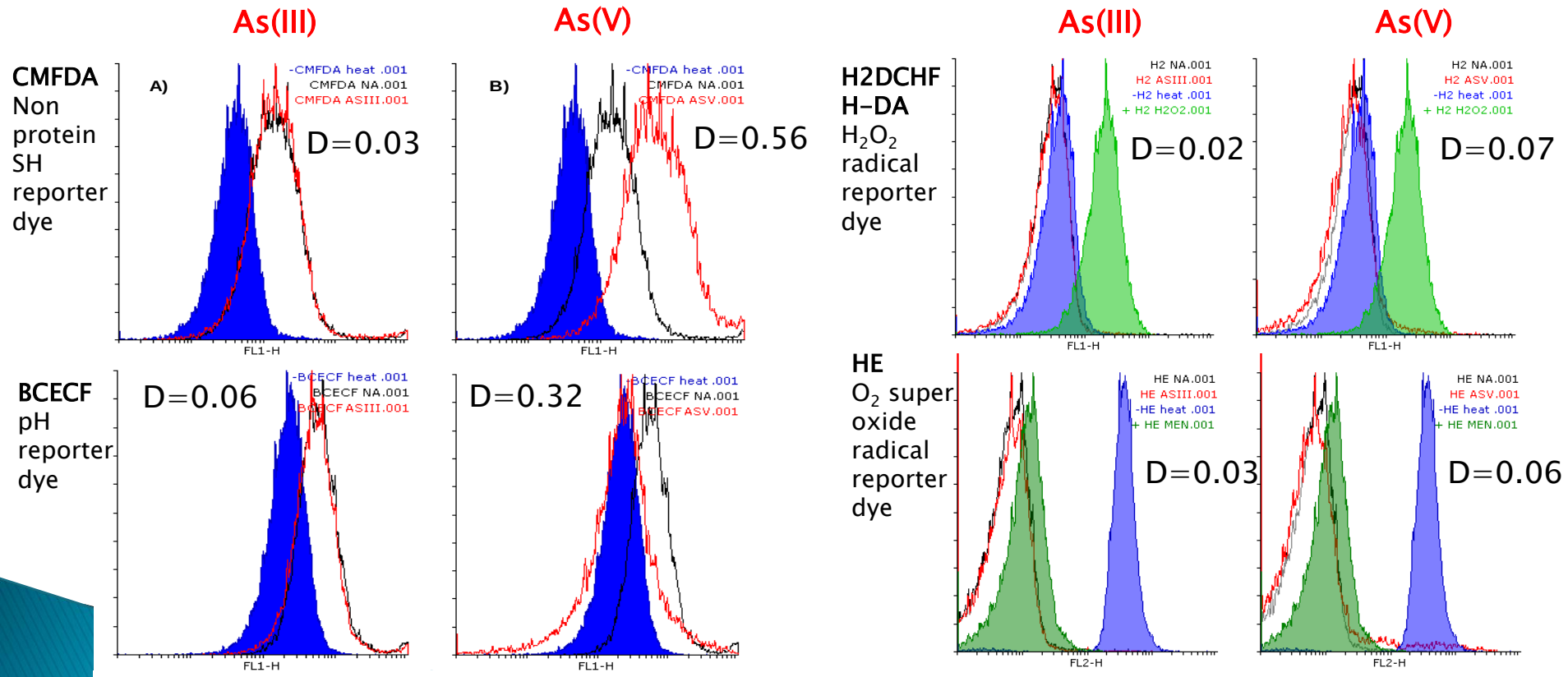
- Toxicity of As(III) (72 h @ 0.3mg $L^{-1}$ phosphate) $IC_{50}$=64.23 mg $L^{-1}$
- Toxicity of As(V) (72 h @ 0.3mg $L^{-1}$ phosphate) $IC_{50}$=1.07 mg $L^{-1}$

→ Chlorophyll as surrogate for cell health




n=20,000 cells, Strong D>0.3, Moderate 0.2< D <= 0.3, Weak 0.15< D <=0.20, Negligible D <= 0.15

Kolmogorov-Smirnov statistics

# ENHANCED DETERMINATION OF ARSENIC–PHYTOCHELATIN COMPLEXES IN *C. VULGARIS* USING FOCUSED SONICATION EXTRACTION*

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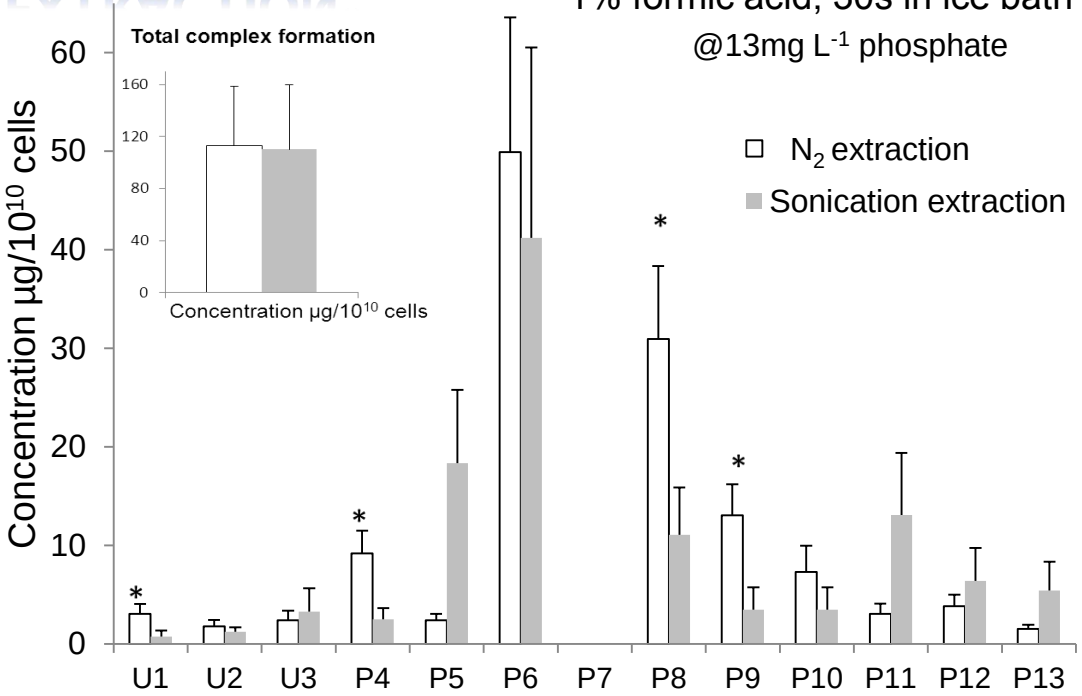
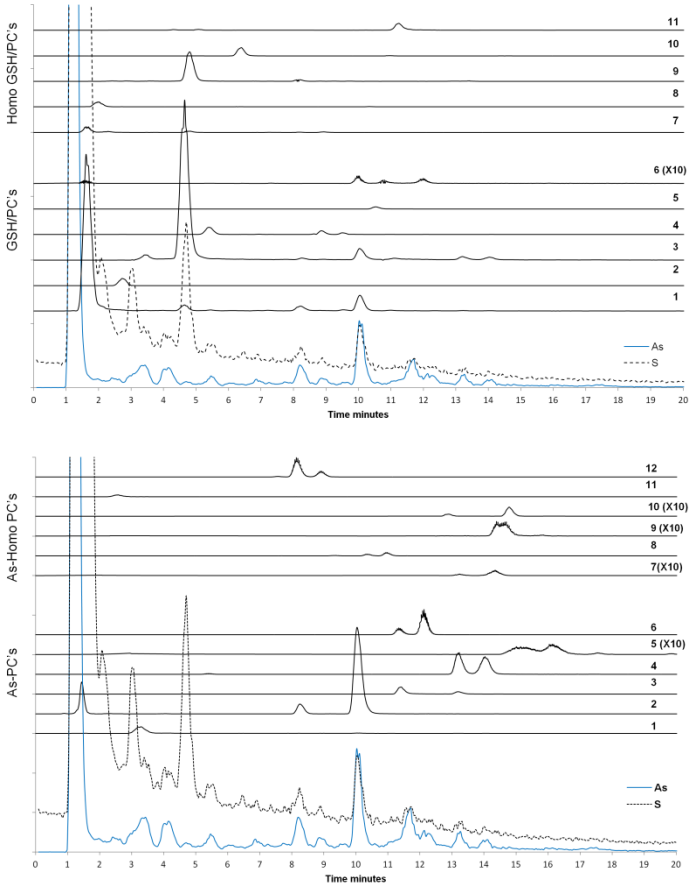
1% formic acid, 30s in ice bath (4˚C)

@13mg $L^{-1}$ phosphate




Online HPLC–ICPMS/ESMS




| Sample | µg As $g^{-1}$ | | % Recovery |
|---|---|---|---|
| Total extraction C. vulgaris | 83.2 | SE = 2.04, n= 6 | |
| Sonication C. vulgaris | 59.2 | SE = 1.14, n = 6 | 71.10% |
| **Quality control** | **µg As $L^{-1}$** | | |
| Total extraction Kelp | 23.5 | SE = 0.60, n = 9 | |
| Sonication Kelp | 22.2 | SE = 0.28, n = 11 | 94.70% |
| SRM 2669 | 48.1 | %RSD = 2.16 | |
| Certifed value | 50.7 | ± 6.3 (95% CI) | |

SE= Standard error , n = Number of samples, % RSD = Relative standard deviation

21 peptides

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# EXPOSURE TO As(III)*

## Cells exposed to 50mg $L^{-1}$ for 48h

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### Unbound peptides

| Molecule | Formula | Monoisotopic mass ($M+H^+$ or $M+2H^+$) | Experimental mass | Difference ppm |
|---|---|---|---|---|
| **GSH/PC** | | | | |
| GSH | $C_{10}H_{17}N_3O_6S$ | 308.0916 | 308.0912 | -1.34 |
| GSSG | $C_{20}H_{32}N_6O_{12}S_2$ | 613.1598 | 613.1598 | 0 |
| Reduced $PC_2$ | $C_{18}H_{29}N_5O10S_2$ | 540.1434 | 540.1433 | -0.16 |
| Oxidised $PC_2$ | $C_{18}H_{27}N_5O_{10}S_2$ | 538.1278 | 538.1289 | 2.04 |
| Reduced $PC_3$ | $C_{26}H_{41}N_7O_{14}S_3$ | 772.1952 | 772.1952 | -0.01 |
| Oxidised $PC_3$ | $C_{26}H_{39}N_7O_{14}S_3$ | 770.1795 | 770.1795 | 0 |
| Reduced $PC_4$ | $C_{34}H_{53}N_9O_{18}S_4$ | 1004.247 | Not found | |
| **Ala GSH/PC** | | | | |
| γ-(Glu-Cys)-Ala | $C_{11}H_{19}N_3O_6S$ | 322.1073 | 322.1072 | -0.21 |
| γ-$(Glu-Cys)_2$-Ala | $C_{19}H_{31}N_5O_{10}S_2$ | 554.1591 | 554.1578 | -2.2 |
| **desGly GSH/PC** | | | | |
| γ-(Glu-Cys) | $C_8H_{14}N_2O_5S$ | 251.0702 | 251.0706 | 1.55 |
| γ-$(Glu-Cys)_2$ | $C_{16}H_{26}N_4O_9S_2$ | 483.1219 | 483.1217 | -0.58 |
| γ-$(Glu-Cys)_3$ | $C_{24}H_{38}N_6O_{13}S_3$ | 715.1737 | 715.1747 | 1.41 |

### Arsenic bound peptides

| Molecule | Formula | Monoisotopic mass ($M+H^+$ or $M+2H^+$) | Experimental mass | Difference ppm |
|---|---|---|---|---|
| **GSH/PC** | | | | |
| As(III)-$PC_2$ | $C_{18}H_{28}N_5O_{11}S_2As$ | 630.0443 | 630.0437 | -0.88 |
| As(III)-$PC_3$ | $C_{26}H_{38}N_7O_{14}S_3As$ | 844.0933 | 844.0931 | -0.19 |
| GS-As(III)-$PC_2$ | $C_{28}H_{43}N_8O_{16}S_3As$ | 460.0666 | 460.0663 | -0.06 |
| | | 919.1253 | 919.1247 | -0.69 |
| As(III)-$(PC_2)_2$ | $C_{36}H_{54}N_{10}O_{20}S_4As$ | 576.0925 | 576.0923 | -0.37 |
| As(III)-$PC_4$ | $C_{34}H_{50}N_9O_{18}S_4As$ | 1076.1451 | 1076.1455 | 0.37 |
| MMA(III)-$PC_2$ | $C_{19}H_{30}N_5O_{10}S_2As$ | 628.0728 | 628.0729 | 0.12 |
| **Ala GSH/PC** | | | | |
| As(III)-γ-$(Glu-Cys)_3$-Ala | $C_{27}H_{40}N_7O_{14}S_3As$ | 858.109 | 858.1082 | -0.87 |
| GS-As(III)-γ-$(Glu-Cys)_2$-Ala | $C_{29}H_{45}N_8O_{16}S_3As$ | 467.0744 | 467.0744 | 0.09 |
| As(III)-γ-$((Glu-Cys)_2)_2$-Ala | $C_{37}H_{57}N_{10}O_{20}S_4As$ | 583.1003 | 583.101 | 1.19 |
| MMA(III)-γ-$(Glu-Cys)_2$-Ala | $C_{20}H_{32}N_5O_{10}S_2As$ | 642.0885 | 642.0889 | 0.66 |
| **desGly GSH/PC** | | | | |
| As(III)-γ-$(Glu-Cys)_2$ | $C_{16}H_{25}N_4O_{10}S_2As$ | 573.0306 | 573.0318 | 2.11 |
| GS-As(III)-γ-$(Glu-Cys)_2$ | $C_{26}H_{40}N_7O_{15}S_3As$ | 431.5558 | 431.5558 | -0.17 |

Glutathione/PC homologues (terminal amino acid Gly substituted by Ala, Ser, Gln, Glu or is absent)

Newly reported peptides

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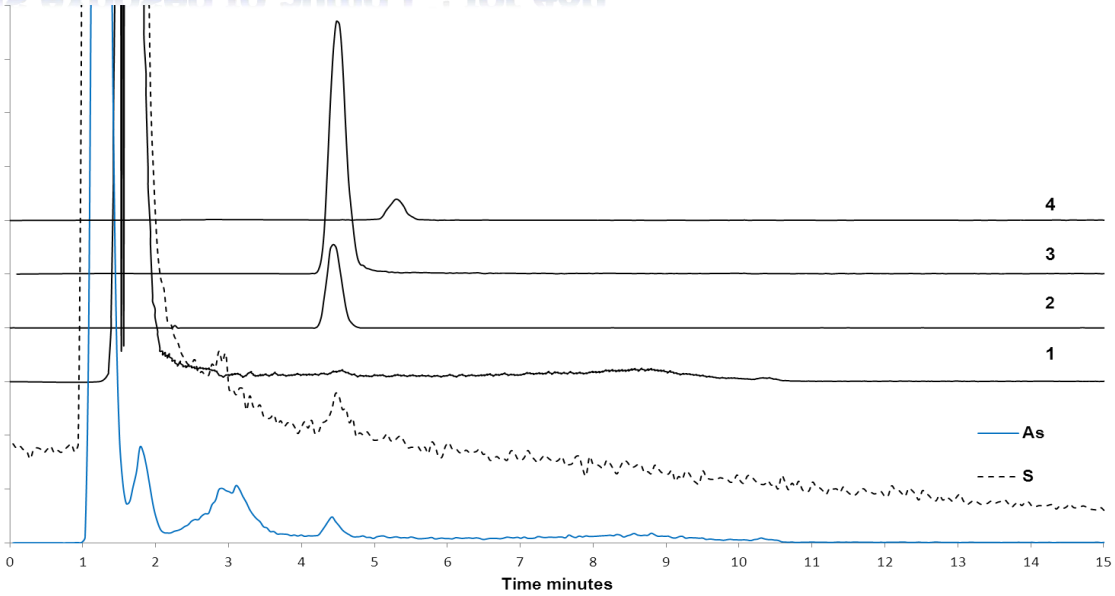
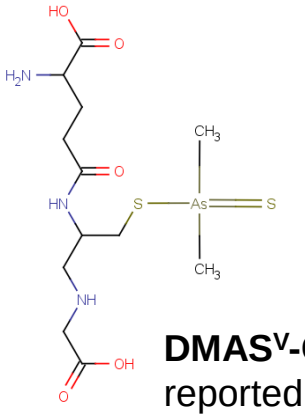
# EXPOSURE TO DMA*

## Cells exposed to 50mg $L^{-1}$ for 48h




**$DMAS^V$-GS** has only been reported once in *Brassica Oleracea* plants:

Cabbage, broccoli, cauliflower, kale, Brussels sprouts, collard greens, savoy, among other

**1** GSH (308), **2 $DMAS^V$-GS (444)**, **3** Reduced $PC_2$ (540) and **4** Oxidised $PC_2$ (538), As and S signals from HR-ICP-MS

@13mg $L^{-1}$ phosphate

| Molecule | Formula (M) | Monoisotopic mass ($M+H^+$) | Experimental mass | Difference ppm |
|---|---|---|---|---|
| GSH | $C_{10}H_{17}N_3O_6S$ | 308.0916 | 308.0918 | 0.65 |
| Red $PC_2$ | $C_{18}H_{29}N_5O_{10}S_2$ | 540.1434 | 540.1433 | -0.16 |
| Ox $PC_2$ | $C_{18}H_{28}N_5O_{10}S_2$ | 538.1278 | 538.1288 | 1.86 |
| $DMAS^V$-GS | $C_{12}H_{23}N_3O_6S_2As$ | 444.0244 | 444.0247 | 0.56 |

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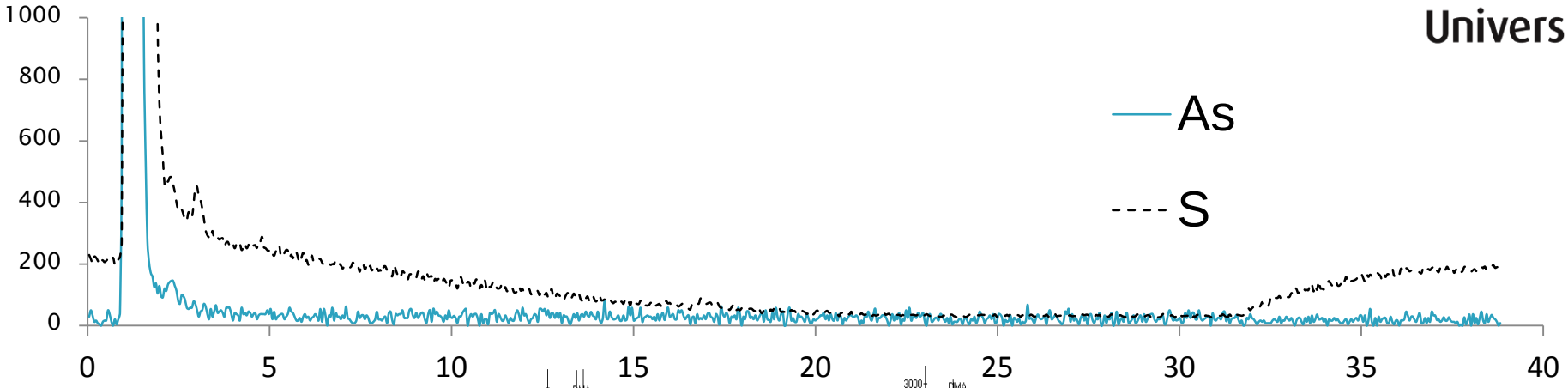
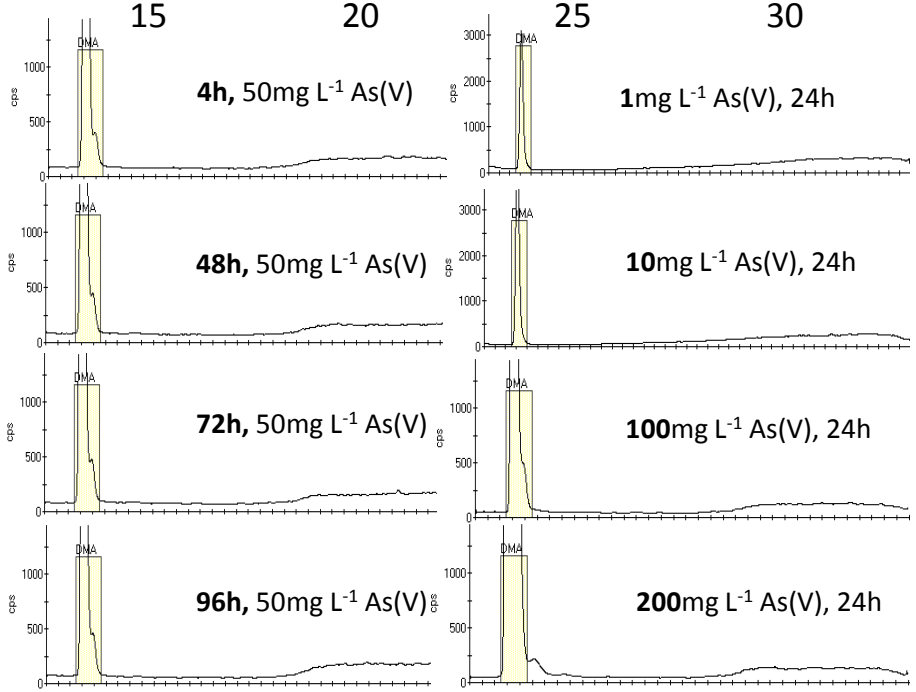
# EXPOSURE TO As(V)*

## Cells exposed to 50mg $L^{-1}$ for 48h




@13mg $L^{-1}$ phosphate

At this level of phosphate cells are not under stress

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# EXPOSURE TO As(III)*

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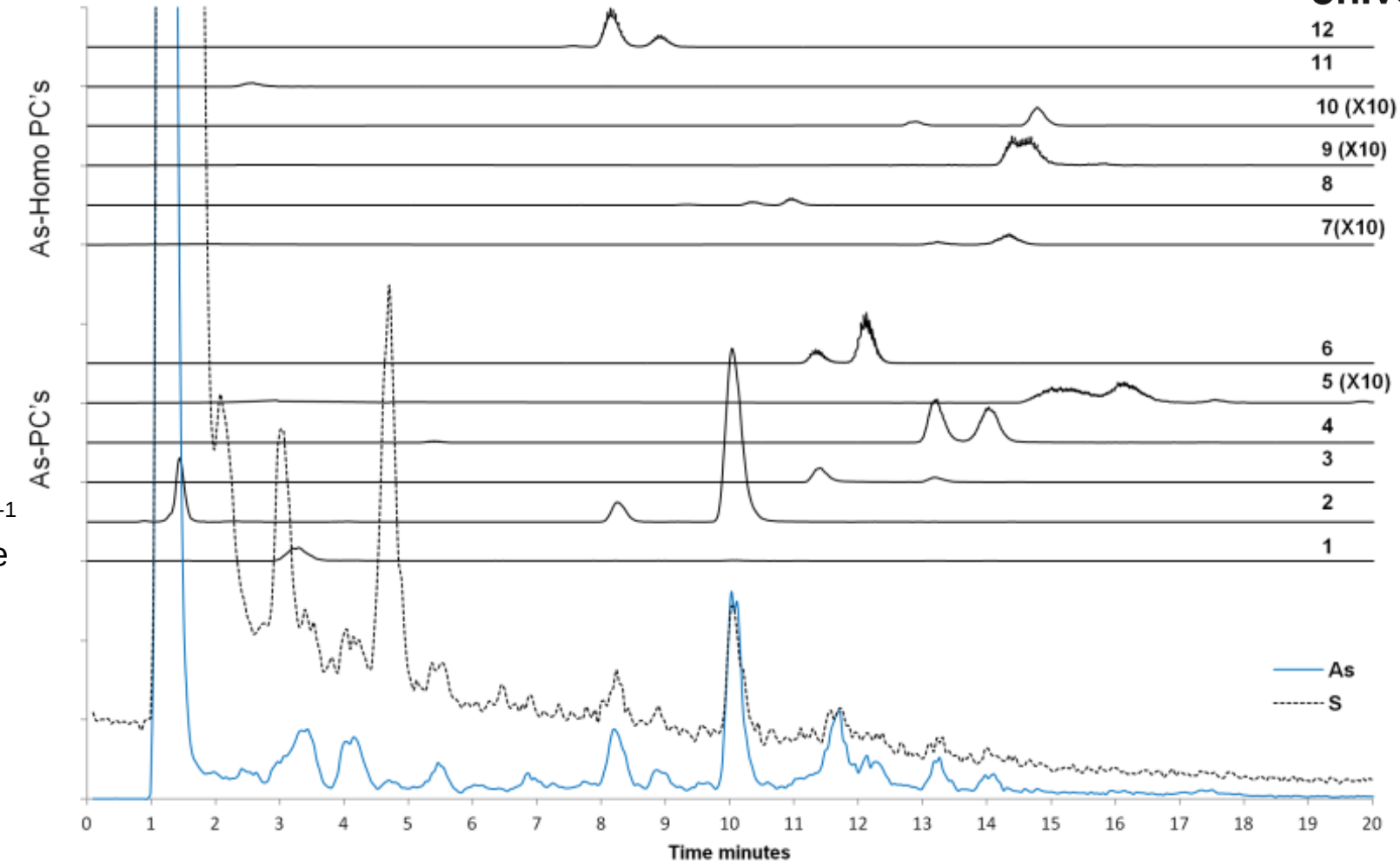


@13mg $L^{-1}$ phosphate

# TOTAL As-GS/PC FORMATION

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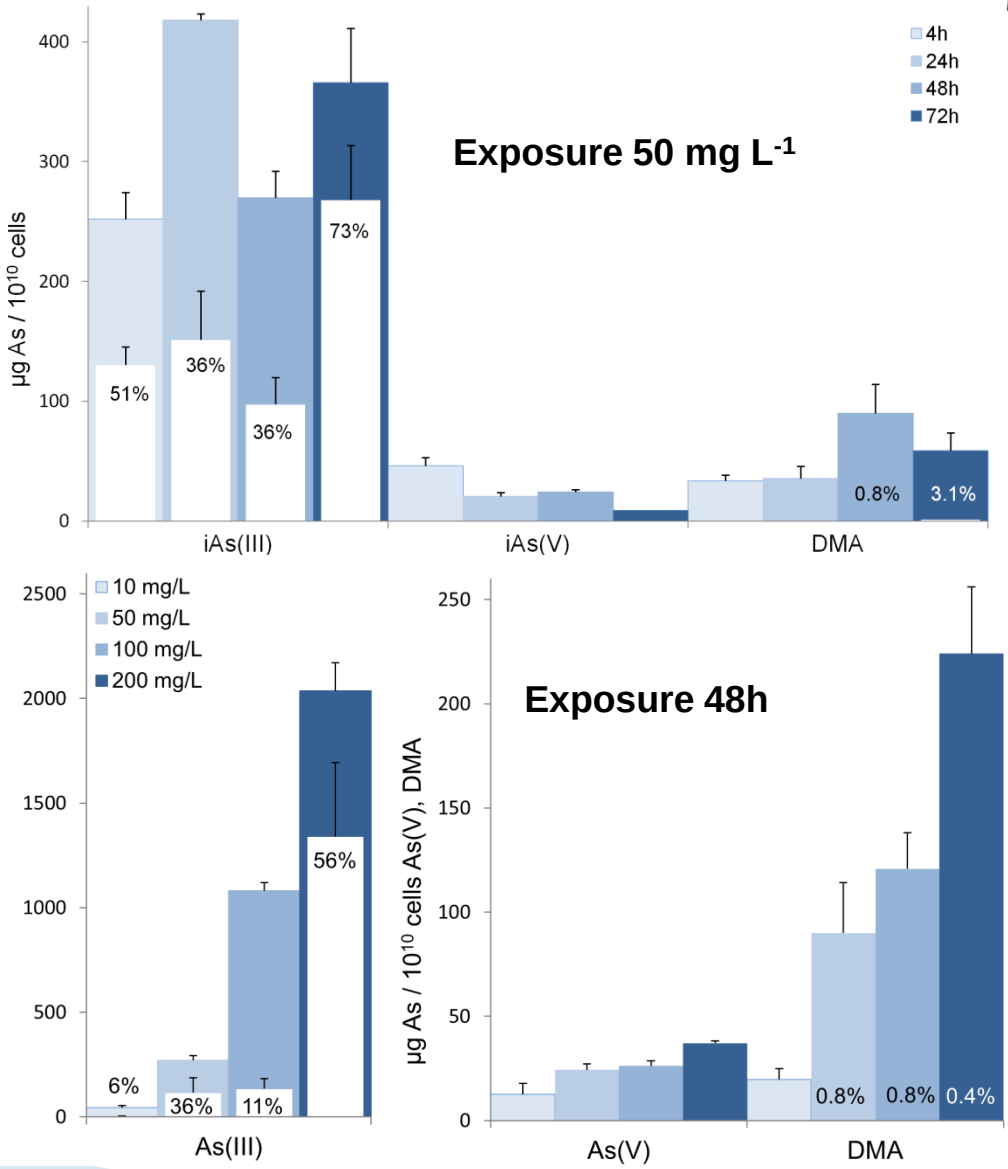
@13mg $L^{-1}$ phosphate

- Coloured bars - total amount of arsenic
- White bars- amount of As-GS/PC (percentage of total arsenic)




Vertical bars denote + 1 standard error, n = 3

# TRANSPORT OF As-GS/PC TO VACUOLES

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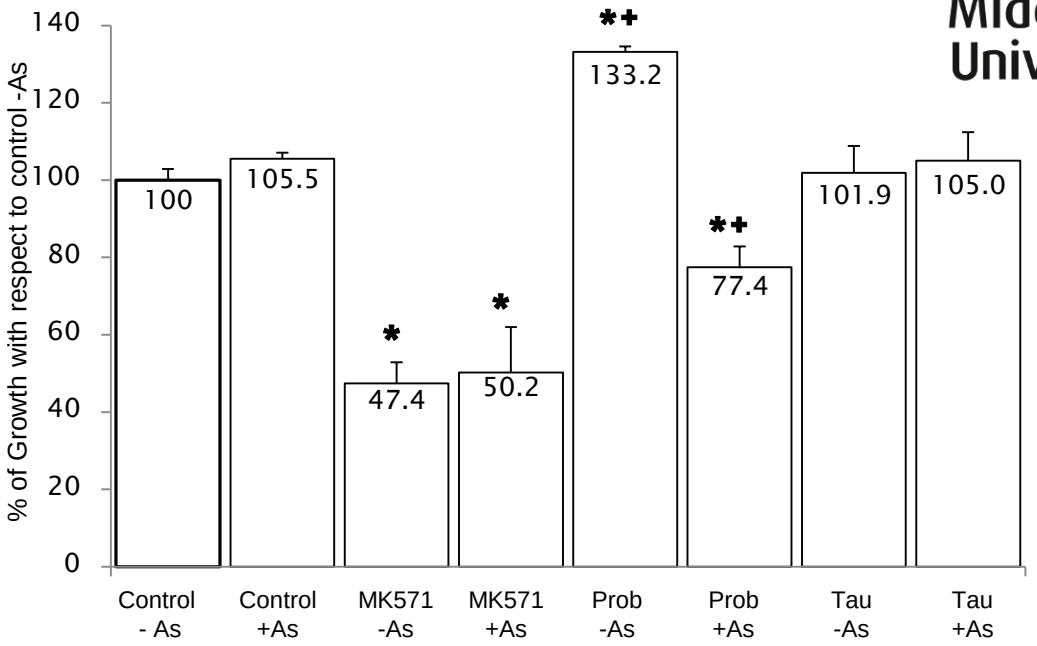
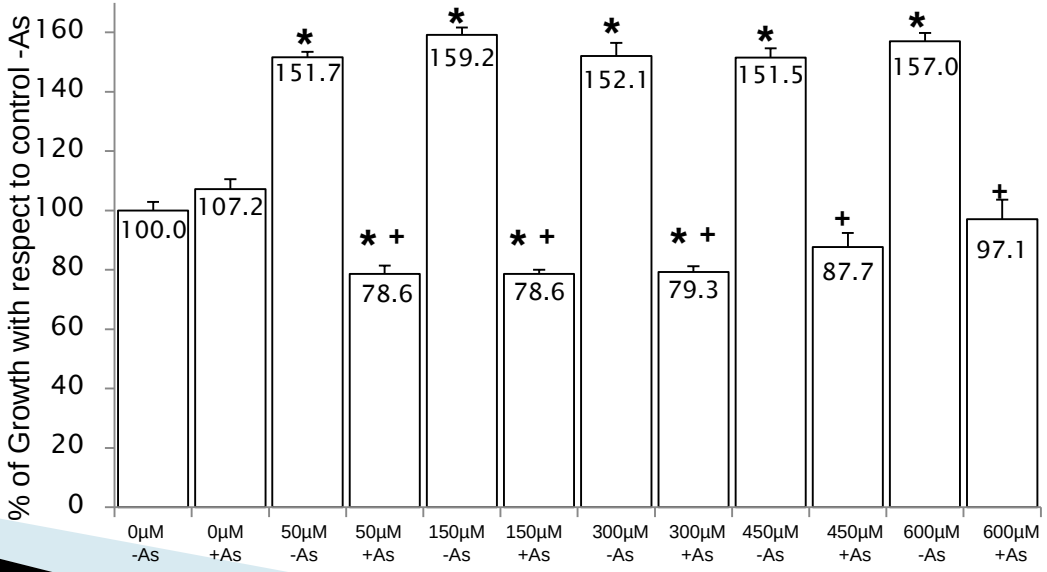
**Toxicity (72 h) to As(III) (50 mg $L^{-1}$)**
@13mg $L^{-1}$ phosphate

Test the presence of ABCC1 and ABCC2 inhibitors:

- MK571(25 μM)
- Probenecid (Prob, 500 μM)
- Sodium taurocholate (Tau, 50 μM).

ABC = ATP-binding cassette transporter
MRP = Multidrug resistance-associated protein




* (P < 0.05) with respect to control
+ (P < 0.05) with respect to absence/presence of arsenic
Vertical bars + 1 standard error
Control n=6, experiments n = 3

# TRANSPORT OF As-GS/PC TO VACUOLES

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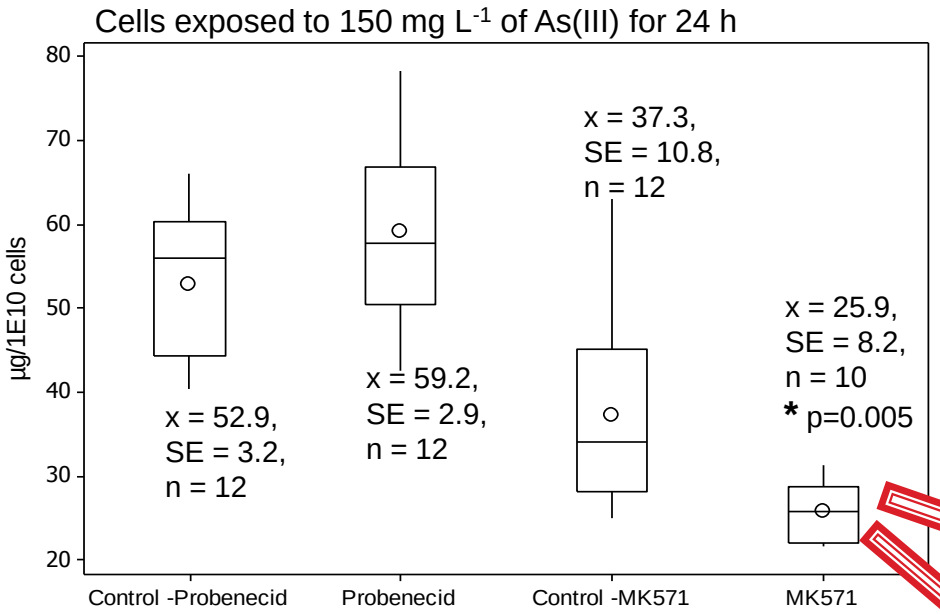
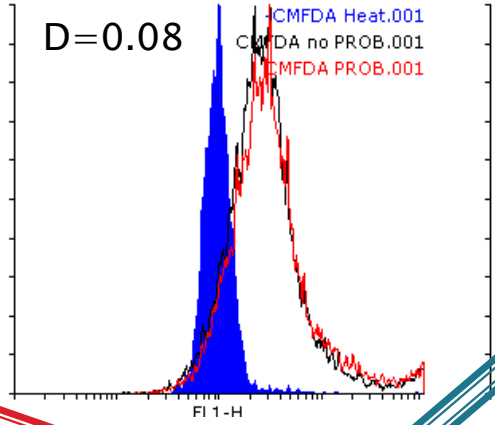
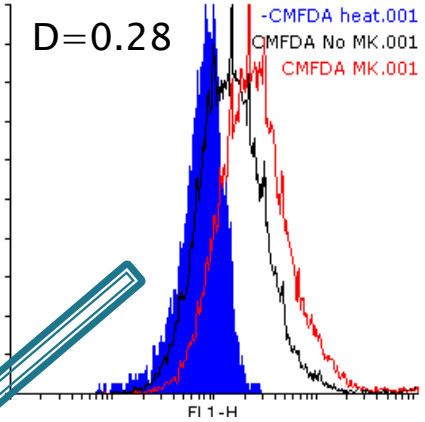



**CMFDA** substrate for ABCC transport

probenecid

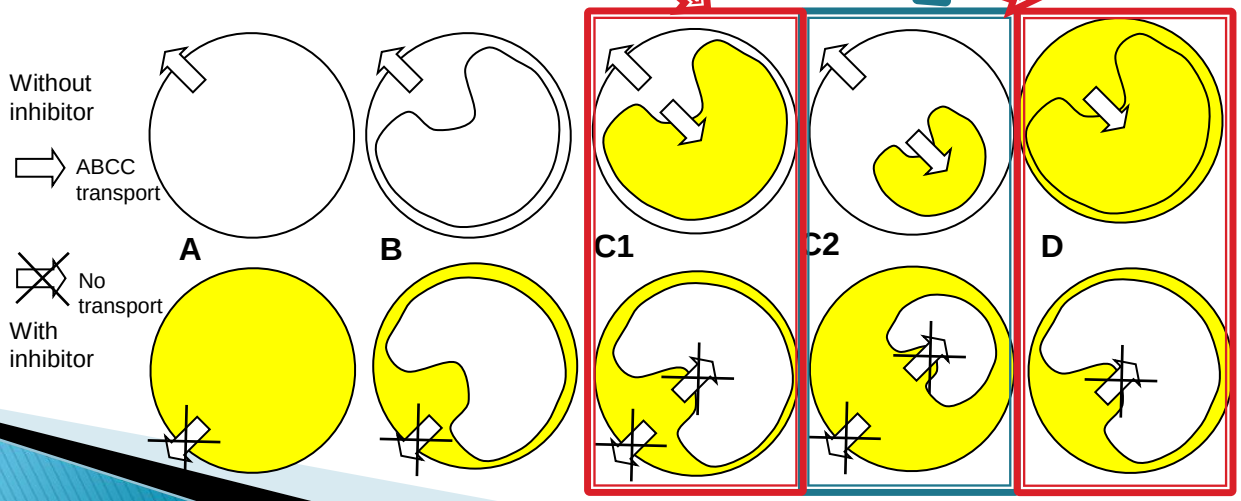



MK571




g D>0.3, Moderate 0.2< D <= 0.3, Weak 0.15< D <=0.20,
Negligib <= 0.15

Treatm me 60 min, n=20,000 cells



# CONCLUSIONS

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- As(V) is more toxic than As(III) to *C. vulgaris* cells at the same concentration of phosphate
- As(III) triggers the formation of **As-GS/PC** molecules
- As(V) does not trigger the formation of **As-GS/PC** molecules when cells are not under stress
- DMA triggers the formation of **$DMAS^{V}$-GS** but it is unlikely that this is part of a detoxification mechanism
- ABCC1 and ABCC2 are involved in **As-GS/P**C transport to acidic vacuoles in *C. vulgaris*

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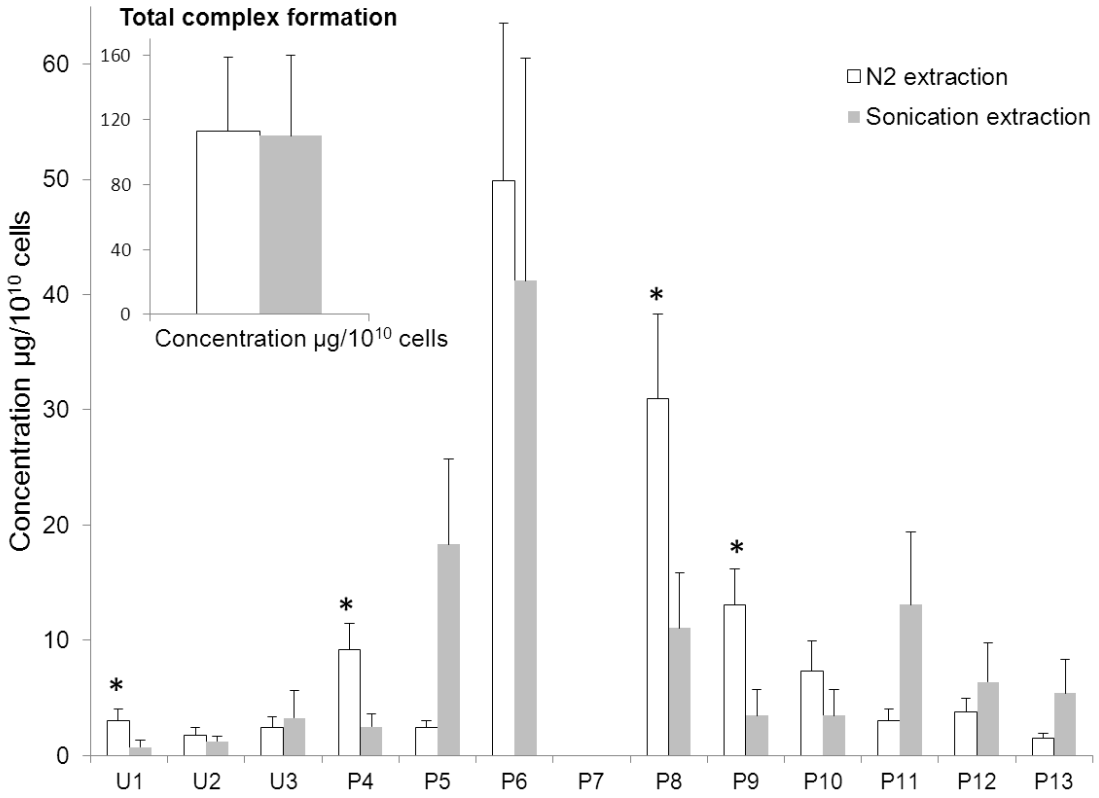
# Thank you for listening.

# Any questions?




| | |
|---|---|
| **U1-3** | Unknowns |
| **P4** | GS-As(III)-$PC_2$/GS-As(III)- $\gamma$-$(Glu\text{-}Cys)_2$, |
| **P5** | As(III)- $\gamma$-$(Glu\text{-}Cys)_2$ |
| **P6** | GS-As(III)-$PC_2$ |
| **P7** | GS-As(III)- $\gamma$-$(Glu\text{-}Cys)_2$-Ala |
| **P8** | As(III)-$PC_3$/ MMA(III)-$PC_2$ |
| **P9** | MMA(III)-$PC_2$ |
| **P10** | As(III)-$PC_3$/ As(III)-$(PC_2)_2$ |
| **P11** | As(III)-$(PC_2)_2$/ As(III)-$\gamma$-$(Glu\text{-}Cys)_3$-Ala/ As(III)- $\gamma$-$((Glu\text{-}Cys)_2)_2$-Ala/ MMA(III)- $\gamma$-$(Glu\text{-}Cys)_2$-Ala, |
| **P12** | As(III)-$PC_4$ |
| **P13** | As(III)-$PC_4$ |